WHAT IS CLAIMED IS:

5

10

15

- 1. A method for evolving a polypeptide and a polynucleotide encoding same by random substitution of nucleotides, comprising the steps of:
- 1) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;
- 2) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;
- 3) inserting a multiple of three substitutive nucleotides into one cut terminus of the target DNA subjected to deletion in Step 2, and deleting the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1;
- 4) subjecting both cut termini of the target DNA obtained in Step 3 to selfligation to obtain a library of mutant DNA having substitutive nucleotides at a random position; and
- 5) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.
- 2. The method of claim 1, wherein Step 2 comprises the steps of introducing a first cassette DNA into the cut position of the target DNA, digesting the cassette DNA with a restriction enzyme, and converting the cut terminus having nucleotides duplicated during the insertion of the transposon to a blunt end, thereby resulting in the deletion of the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon.

3. The method of claim 1, wherein Step 3 comprises the steps of introducing a second cassette DNA containing a multiple of three consecutive substitutive nucleotides into the cut position of the DNA obtained in Step 2, digesting the second cassette DNA with a restriction enzyme, and converting both cut termini of the resulting DNA fragment to blunt ends, thereby resulting in the addition of the substitutive nucleotides into one cut terminus of the target DNA subjected to deletion in Step 2 and the deletion of the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1.

10

- 4. The method of claim 1, wherein the transposon is selected from the group consisting of Tn4430, Tn7, mini-Mu and derivatives thereof.
- 5. The method of claim 1, wherein the substitutive nucleotides introduced in Step 3 have a specific nucleotide sequence.
 - 6. The method of claim 1, wherein the substitutive nucleotides introduced in Step 3 have a random nucleotide sequence.
- 7. The method of any one of claims 1 to 6, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins
- 8. The method of claim 7, wherein the enzyme is selected from the group consisting of hydrolase, lyase, transferase, oxidoreductase, ligase and isomerase.
 - 9. The method of claim 2 or 3, wherein the restriction enzyme is a class IIS restriction enzyme.

- 10. A method for evolving a polypeptide and a polynucleotide encoding same by random insertion of nucleotides, comprising the steps of:
- 1) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;
- 2) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;
- 3) inserting a multiple of three additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step 2, and deleting the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step 1;
- 4) subjecting both cut termini of the target DNA obtained in Step 3 to selfligation to obtain a library of mutant DNA having additional nucleotides at a random position; and
- 5) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

20

25

30

5

10

- 11. The method of claim 10, wherein Step 2 comprises the steps of introducing a first cassette DNA into the cut position of the target DNA, digesting the cassette DNA with a restriction enzyme, and converting the cut terminus having nucleotides duplicated during the insertion of the transposon to a blunt end, thereby resulting in the deletion of the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon.
- 12. The method of claim 10, wherein Step 3 comprises the steps of introducing a second cassette DNA containing a multiple of three consecutive additional nucleotides into the cut position of the DNA obtained in Step 2, digesting the second

cassette DNA with a restriction enzyme, and converting both cut termini of the resulting DNA fragment to blunt ends, thereby resulting in the insertion of the additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step 2, and the deletion of the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step 1.

5

15

25

- 13. The method of claim 10, wherein the transposon is selected from the group consisting of Tn4430, Tn7, mini-Mu and derivatives thereof.
- 10 14. The method of claim 10, wherein the additional nucleotides introduced in Step 3 have a specific nucleotide sequence.
 - 15. The method of claim 10, wherein the additional nucleotides introduced in Step 3 have a random nucleotide sequence.
 - 16. The method of any one of claims 10 to 15, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins
- 20 17. The method of claim 16, wherein the enzyme is selected from the group consisting of hydrolase, lyase, transferase, oxidoreductase, ligase and isomerase.
 - 18. The method of claim 11 or 12, wherein the restriction enzyme is a class IIS restriction enzyme.
 - 19. A method for evolving a polypeptide and a polynucleotide encoding same by random deletion of nucleotides, comprising the steps of:
 - 1) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites

to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

2) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1;

5

10

15

20

25

- 3) subjecting both cut termini of the target DNA obtained in Step 2 to selfligation to obtain a library of mutant DNA having a deletion of nucleotides at a random position; and
- 4) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.
- 20. The method of claim 19, wherein Step 2 comprises the steps of introducing a cassette DNA into the cut position of the target DNA, digesting the cassette DNA with a restriction enzyme, and converting both cut termini of the resulting DNA fragment to blunt ends, thereby resulting in the deletion of the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the deletion of the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1.
- 21. The method of claim 19, wherein the transposon is selected from the group consisting of Tn4430, Tn7, mini-Mu and derivatives thereof.
- 22. The method of any one of claims 19 to 21, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins

- 23. The method of claim 22, wherein the enzyme is selected from the group consisting of hydrolase, lyase, transferase, oxidoreductase, ligase and isomerase.
- 24. The method of claim 20, wherein the restriction enzyme is a class IIS restriction enzyme.

5

10

15

- 25. A method for evolving a polypeptide and a polynucleotide encoding same, comprising the steps of:
- 1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by at least one of the methods of claims 1, 10 and 19, into a target polynucleotide; and
- 2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.
- 26. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of any one of claims 1, 10 and 19 with the mutant polynucleotide prepared by the method of claim 25 as a target polynucleotide.